conditions, ARF-BP1/Mule does not downregulate p53 and Mcl-1 simultaneously. The biological and molecular principles that guide the selective choice of targets by ARF-BP1 may therefore hold the key to the enigma.

Owing to its ability to control the levels of key molecules such as p53 and Mcl-1, ARF-BP1/Mule is likely to be important in cell fate determination. Its large size suggests that, in addition to the HECT E3 domain and the structural motifs mediating binding of p53 and Mcl-1, ARF-BP1/Mule may hide numerous additional molecular secrets. Future work should unravel those secrets and enable the proper positioning of ARF-BP1/Mule within the highly dynamic network of signals that underpins cell fate decisions.

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Selected Reading

Cans, C., Passer, B.J., Shalak, V., Nancy-Portebois, V., Crible, V., Amzallag, N., Allanic, D., Tufino, R., Argentini, M., Moras, D., et al. (2003). Proc. Natl. Acad. Sci. USA *100*, 13892–13897.

Chen, D., Kon, N., Li, M., Zhang, W., Qin, J., and Gu, W. (2005). Cell *121*, this issue, 1071–1083.

Dornan, D., Wertz, I., Shimizu, H., Arnott, D., Frantz, G.D., Dowd, P., O'Rourke, K., Koeppen, H., and Dixit, V.M. (2004). Nature *429*, 86–92.

Hershko, A., and Ciechanover, A. (1998). Annu. Rev. Biochem. *67*, 425–479.

Leng, R.P., Lin, Y., Ma, W., Wu, H., Lemmers, B., Chung, S., Parant, J.M., Lozano, G., Hakem, R., and Benchimol, S. (2003). Cell *112*, 779–791.

Liu, H., Peng, H.W., Cheng, Y.S., Yuan, H.S., and Yang-Yen, H.F. (2005). Mol. Cell. Biol. *25*, 3117–3126.

Lowe, S.W., and Sherr, C.J. (2003). Curr. Opin. Genet. Dev. *13*, 77–83.

Michels, J., Johnson, P.W., and Packham, G. (2005). Int. J. Biochem. Cell Biol. *37*, 267–271.

Oren, M. (2003). Cell Death Differ. *10*, 431–442.

Zhong, Q., Gao, W., Du, F., and Wang, X. (2005). Cell *121*, this issue, 1085–1095.

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A Disulfide Relay System in Mitochondria

In this issue of *Cell*, Mesecke et al. (2005) show that there is a disulfide relay system in the intermembrane space (IMS) of mitochondria that is comprised of the proteins Mia40 and Erv1. This disulfide relay system promotes the import and oxidative folding of proteins. Oxidized Mia40 traps newly imported proteins through mixed disulfide bridges. Subsequent isomerization of these disulfide bridges allows the imported protein to be folded in the IMS. The reduced Mia40 generated is then reoxidized by the sulfhydryl oxidase Erv1, promoting the next round of disulfide exchange. The new work clarifies the molecular function of Mia40 and reveals Mia40 to be the first physiological substrate for the FAD-linked Erv1.

All proteins of the mitochondrial intermembrane space (IMS) are synthesized in the cell cytosol and are then imported into the mitochondria. Most of them do not contain any cleavable sequences and are targeted to the mitochondrial IMS by as yet unidentified import signals. The IMS import pathway differs from that of proteins targeted to the mitochondrial inner membrane or matrix because import of proteins to the IMS does not require ATP or an inner membrane electric potential. It is generally thought that folding of these proteins in the IMS, often facilitated by cofactor binding or association with multiprotein complexes, may provide a mechanism for retaining these proteins in mitochondria. This is particularly important for proteins that exist in both the cytosol and the IMS.

It has recently been found that Mia40 (also called Tim40) is the first component of an IMS-specific protein import pathway (Chacinska et al., 2004; Naoe et al., 2004; Terziyska et al., 2005) (see Figure 1). Yeast Mia40 is an essential gene with homologs in fungi, plants, and animals. Depletion of Mia40 or abrogation of its function through mutation block the import of small cysteine-rich IMS proteins (such as members of the small Tim family and Cox17) but not of proteins targeted to the mitochondrial matrix or the inner membrane. Mia40 binds transiently to precursor proteins imported into the IMS, facilitating their passage across the outer membrane and their retention in the IMS. Mia40 has six conserved cysteines (four of which form a duplicate CX9C motif) that are essential for its function (Naoe et al., 2004), although their exact role is unknown. The cysteines of Mia40 have been proposed to interact with precursor protein substrates imported into the IMS because the amount of substrate accumulated in Mia40 complexes decreases in the presence of reducing agents but is stabilized by $CuCl_2$, which induces cross-linking of disulfide bonds (Chacinska et al., 2004).

In the new work, Mesecke et al. (2005) develop this point further with their demonstration that Mia40 forms a transient intermediate with imported precursor proteins via an intermolecular disulfide bond. Subsequent isomerization of this disulfide bond results in oxidation of the imported precursor protein, which now contains an intramolecular disulfide bond and can proceed with folding. Using Tim13 (a protein containing the CX3C motif) and Cox17 (a protein containing the CX9C motif) as substrates, the authors show that radioactively tagged precursor proteins or purified Tim13 and Cox17 can be linked to Mia40 via DTT-sensitive disulfide bridges (mixed disulfides). Furthermore, conditions that keep Mia40 in a reduced state seem to block the import of Tim13 and Cox17 into the IMS. In addition, high concentrations of DTT also blocked the import of Tim13 and Cox17 both in vivo and in vitro. In contrast, low concentrations of DTT (below 5 mM) actually boosted the import of Tim13 and Cox17, in agreement with a previous study by Lu et al. (2004a) showing that oxida-

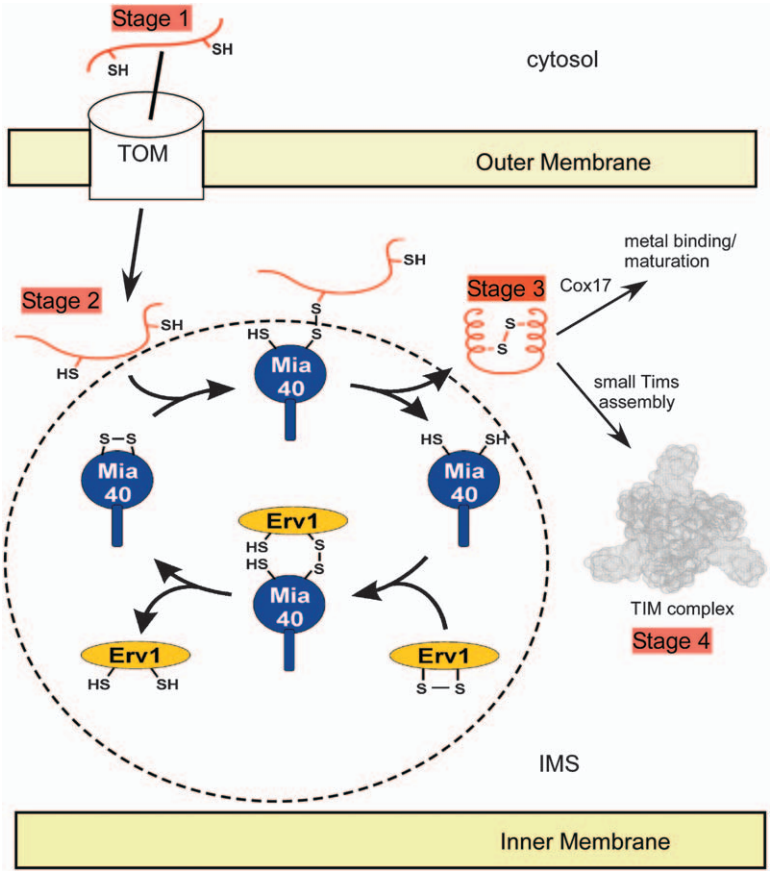

Figure 1. Import and Folding of Cysteine-Rich Proteins in the Intermembrane Space of Mitochondria

Central to the import and folding of cysteine-rich proteins in the mitochondrial IMS is the Mia40/Erv1 redox cycle (circle) involving a disulfide bond relay system. The precursor protein to be imported into the IMS first is reduced in the cytosol and then is translocated through the TOM channel into the mitochondria in its reduced form. The reduced protein forms a transient intermolecular disulfide bridge with the oxidized form of Mia40. This results in oxidation of the precursor protein that now contains an intramolecular disulfide bond and is able to undergo folding in the IMS. The precursor protein may need to undergo repeated cycles of oxidation and disulfide bond isomerization to ensure proper folding. Protein folding may involve additional pathways, for example, in the case of Cox17 binding to metals or in the case of the Tim proteins, assembly of an hetero-oligomer composed of oxidized subunits (shown here as the SAXS structure of the TIM10 complex; Lu et al., 2004b).

tion prior to import blocks translocation through the TOM channel.

Mia40 can also bind to metals (Terziyska et al., 2005; Chacinska et al., 2004), which may be relevant for the maturation of some proteins in the IMS. In this respect, Mia40 may have a similar function to CCS, the chaperone that brings copper to superoxide dismutase (SOD1). CCS forms mixed disulfide bonds with SOD1 upon its import as an apoprotein (Field et al., 2003). Subsequent isomerization of the mixed disulfide bonds to form an intramolecular SOD1 disulfide bond result in binding of copper and release of SOD1 in its folded conformation (Furukawa et al., 2004).

To regenerate its active form after donating a disulfide bond to the incoming precursor protein, Mia40 needs to become reoxidized in the IMS. But how does this happen? Mesecke and colleagues address this conundrum by fingering the FAD-linked sulfhydryl oxidase, Erv1 (Lee et al., 2000). So far, no substrates have been identified for Erv1 in vivo. First, the authors show that in Erv1-depleted cells, there is a marked reduction in the amount of IMS proteins. Second, using an Mia40-His-tagged protein and a coimmunoprecipitation assay, the investigators discovered a direct interaction between Mia40 and Erv1. Third, they report that in Erv1-depleted cells, Mia40 seems to remain in a reduced state. Conversely, when Erv1 is overexpressed, there is an increase in the amount of oxidized Mia40. Here, a word of caution is probably warranted. The authors assessed the presence of the reduced or oxidized form of Mia40 through the differential migration of the two forms on a gel. A previous study suggests that Mia40 may migrate aberrantly in gels due to its highly acidic pI (Chacinska et al., 2004). To confirm this point and to gain a better understanding of the disulfide bonds in Mia40, more detailed studies with thiol-trapping methods are needed. Collectively, however, the new data point to the exciting conclusion that Mia40 is a physiological substrate of the sulfhydryl oxidase Erv1.

The Mesecke et al. study is of major interest for two reasons to those studying the import into the IMS of proteins containing conserved cysteines. First is the fact that Mia40 donates at least one disulfide bond to the incoming precursor protein. Second is the notion that Erv1 plays a key role in the oxidation cycle of Mia40, thus linking this essential FAD-linked oxidase to the import process. Earlier studies had already proposed an oxidative folding pathway in mitochondria (Lu et al., 2004a; Lu et al., 2004b). The data of Mesecke and coworkers provide direct support for this notion by defining Mia40 as the oxidizing component of this pathway.

The Mesecke et al. study sets the stage for a more complete understanding of the redox-dependent import pathway of mitochondria. However, several important questions remain. What happens downstream of Erv1 oxidation of Mia40, that is, what is the final electron acceptor for this oxidative folding pathway? It

is tempting to speculate that, as in the bacterial periplasm, the final acceptor may be the chain of respiratory proteins, but this is speculation. Does Mia40 randomly oxidize any free sulphydryl groups on the imported precursor protein substrates? How can the correct disulfide bonds form in this case? Is there a distinct isomerase function (with no net electron transfer) associated with the Mia40 oxidation-reduction cycle? If so, how are additional disulfide bonds formed? For cases such as the small Tim proteins that must maintain all of their cysteines in an oxidized state to ensure correct assembly (Lu et al., 2004a; Curran et al., 2002), it is conceivable that once the first disulfide bond is formed, formation of the second disulfide bond is facilitated by juxtaposition of the two remaining cysteines (Allen et al., 2003). An electron acceptor is still necessary for this second oxidation step, but whether it turns out to be Mia40 remains an open question. Hot13 has also been suggested to influence the assembly of small Tim proteins (Curran et al., 2004), but how is this relevant to the Mia40/Erv1 cycle? Mesecke et al. propose that Hot13 may operate in an as yet undiscovered reductive pathway, but its exact role awaits elucidation. It is still unclear what happens after Mia40-mediated oxidation of the imported substrate and how the substrate is released. Indeed, different substrates may have different fates. For example, Cox17 exists as two conformers (Arnesano et al., 2005): in one, copper is bound and in addition there are two disulfide bonds, whereas the other is an oligomeric copper conformer containing no disulfide bonds. However, it is still unclear which of the two conformers is the physiological form of Cox17. Finally, the need for Erv1 in the maturation of cytosolic proteins containing Fe-S clusters (Lange et al., 2001) raises the intriguing possibility that the disulfide relay system is not limited to the import and folding of IMS proteins but rather may also be involved in the export of Fe-S cluster proteins from the mitochondrial matrix to the cell cytosol.

Given the evolutionary relationship of the mitochondrial IMS to the bacterial periplasm, one cannot help but draw parallels between the function of mitochondrial Erv1 and its bacterial counterpart dsbB, which is functionally related to Erv2 of the eukaryotic endoplasmic reticulum. Erv1 oxidizes Mia40, and dsbB oxidizes dsbA. These proteins in turn oxidize translocated substrates. The new findings may relieve some of the initial skeptisicm about the idea of oxidative protein folding in mitochondria. This idea is, after all, counterintuitive given the fact that the mitochondrial IMS is thought to be in equilibrium with the cell cytosol, with free exchange of metabolites between the IMS and cytosol via porin channels. However, defining specific redox partners, such as Erv1, Mia40, and possibly Hot13, should help to elucidate further the mechanisms involved in oxidative protein folding. We have identified just the tip of the iceberg of an unexpected mechanism that may be crucial for the physiological response of cells to redox conditions.

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Selected Reading

Arnesano, A., Balatri, E., Banci, L., Bertini, I., and Winge, D. (2005). Structure *13*, 713–722.

Allen, S., Lu, H., Thornton, D., and Tokatlidis, K. (2003). J. Biol. Chem. *278*, 38505–38513.

Chacinska, A., Pfannschmidt, S., Wiedemann, N., Kozjak, V., Sanjuan Szklarz, L.K., Schulze-Specking, A., Truscott, K.N., Guiard, B., Meisinger, C., and Pfanner, N. (2004). EMBO J. *23*, 3735–3746.

Curran, S.P., Leuenberger, D., Oppliger, W., and Koehler, C.M. (2002). EMBO J. *21*, 942–953.

Curran, S.P., Leuenberger, D., Leverich, E.P., Hwang, D.K., Beverly, K.N., and Koehler, C.M. (2004). J. Biol. Chem. *279*, 43744–43751.

Field, L.S., Furukawa, Y., O'Halloran, T.V., and Culotta, V.C. (2003). J. Biol. Chem. *278*, 28052–28059.

Furukawa, Y., Torres, A.S., and O'Halloran, T.V. (2004). EMBO J. *23*, 2872–2881.

Lange, H., Lisowsky, T., Gerber, J., Muhlenhoff, U., Kispal, G., and Lill, R. (2001). EMBO Rep. *8*, 715–720.

Lee, J.-E., Hofhaus, G., and Lisowsky, T. (2000). FEBS Lett. *477*, 62–66.

Lu, H., Allen, S., Wardleworth, L., Savory, P., and Tokatlidis, K. (2004a). J. Biol. Chem. *279*, 18952–18958.

Lu, H., Golovanov, A.P., Alcock, F., Grossmann, J.G., Allen, S., Lian, L.Y., and Tokatlidis, K. (2004b). J. Biol. Chem. *279*, 18959–18966.

Mesecke, N., Terziyska, N., Kozany, C., Baumann, F., Neupert, W., Hell, K., and Hermann, J. (2005). Cell *121*, this issue, 1059–1069.

Naoe, M., Ohwa, Y., Ishikawa, D., Ohshima, C., Nishikawa, S., Yamamoto, H., and Endo, T. (2004). J. Biol. Chem. *279*, 47815–47821.

Terziyska, N., Lutz, T., Kozany, C., Mokranjac, D., Mesecke, N., Neupert, W., Herrmann, J.M., and Hell, K. (2005). FEBS Lett. *579*, 179–184.

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